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Serotomide and safflomide modulate forskolin-stimulated cAMP formation via 5-HT1 receptor

J.B. Park*

Diet, Genomics, and Immunology Laboratory, Bldg. 307C, Rm. 131, BHNRC, ARS, USDA, Beltsville, MD 20705, USA

Abstract

Serotomide (trans-*N*-caffeoylserotonin) and safflomide (trans-*N*-caffeoyltryptamine) belong to serotonin-derived phenylpropenoid amides found in plants. In this paper, serotomide and safflomide were investigated to determine their effects on serotonin receptor 5-HT1 in the renal epithelial (OK) cells, due to their structural similarity to 5-HT1 receptor ligands. At the concentration of 10 μM, serotomide was able to inhibit forskolin-stimulated cAMP formation in the OK cells by 31% ($p<0.019$). The inhibition was repressed by Nan-190 and spiperone (5-HT1 antagonists), suggesting that serotomide suppresses cAMP formation via binding to 5-HT1 receptors in the OK cells. Meanwhile, safflomide could not inhibit forskolin-stimulated cAMP formation at the same concentration (10 μM), but repress the inhibition of forskolin-stimulated cAMP by serotonin agonists (e.g., serotonin and 8-OH-DPAT) by 31% ($p<0.018$), suggesting that safflomide may block 5-HT1 receptors in a similar way to Nan-190 and spiperone. All together the data indicate that serotomide and safflomide may be potent compounds that respectively act to activate and to block 5-HT1 receptors on OK cells.
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Keywords: Serotomide; Safflomide; Coffee; 5-HT1 receptor; cAMP; Renal epithelial (OK) cells; Forskolin

Introduction

Serotomide (trans-*N*-caffeoylserotonin) and safflomide (trans-*N*-caffeoyltryptamine) belong to serotonin-derived phenylpropenoid amides produced in plants via forming an amide bond between the carboxyl group of phenylpropenoic acid and the amine groups of serotonin derivatives (Fig. 1) (Akhondzadeh et al., 2007; Jang et al., 2004; Kang et al., 2006; Sarker et al., 2001; Niwa et al., 2000). The phenylpropenoid amides are found in several plants, including *Coffea canephora*, *Theobroma cacao*, *Amorphophallus konja*, *Ipomoea obscura* and *Carthamus tinctorius* (Niwa et al., 2000; Stark et al., 2006; Jenett-Siems et al., 2003; Zhang et al., 1997). Recent studies suggest that safflomide and its analogues may have numerous biological activities implicated in preventing and/or treating several human chronic diseases such as inflammation, atherosclerosis and others (Park and Chen, 2007; Ohnishi et al., 1998; Roh et al., 2004; Koyama et al., 2006; Takii et al., 2003). However, little is known about the effects of serotomide and safflomide on the human serotonin (5-HT) receptors, even though serotomide and safflomide have serotonin-derived chemical structures.

Serotonin (5-HT) receptor family consists of seven main families (5-HT1–5-HT7) (Lanfumey and Hamon, 2004; Leysen, 2004; Thompson and Lummis, 2006; Hegde and Eglen, 1996; Nelson, 2004; Woolley et al., 2004; Thomas and Hagan, 2004). Particularly, 5-HT1 receptors are widely distributed in the central nervous system and other tissues, implicated in pathophysiological processes

*Tel.: +1 301 504 8365; fax: +1 301 504 9456.
E-mail address: jae.park@ars.usda.gov.

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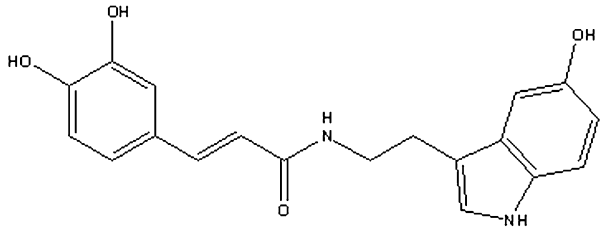
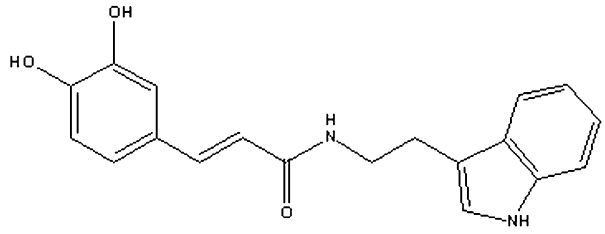


Serotomide



Safflomide

Fig. 1. Chemical structures of serotomide (trans-*N*-caffeoyl-serotonin) and safflomide (trans-*N*-caffeoyltryptamine).

of several human diseases such as depression, anxiety, migraine, and cognitive dysfunction (Lanfumey and Hamon, 2004). Due to the physiological importance of 5-HT1 receptors, their agonists and antagonists have been explored and developed in order to treat human diseases associated with 5-HT1 receptors. Interestingly, serotomide and safflomide have chemical structures similar to 5-HT1 receptor ligands. Therefore, in this paper, the effects of safflomide and serotomide on cAMP production were investigated in renal epithelial OK because of the link between 5-HT1 receptors and G alpha i/G alpha o proteins that regulate adenylyl cyclase activity (Lanfumey and Hamon, 2004; Malmberg and Strange, 2000). Also, potential beneficial effects of serotomide and safflomide on human health were discussed in this paper.

Materials and methods

Materials

Forskolin and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Renal epithelial (OK) cell line was purchased from ATCC (Manassas, VA).

Serotomide and safflomide

Serotomide was prepared via the synthesis according to the procedures described previously (Park and Chen, 2007; Park and Schoene, 2002; Park, 2005). Briefly, caffeic acid was dissolved in dichloromethane and converted to the symmetrical anhydride with 1,3-diisopropylcarbodiimide. Serotonin was added to the reaction mixture, and then stirred gently for 8 h at room temperatures. The synthesized serotomide was further purified by HPLC (Waters, Milford, MA). Safflomide was also prepared as described previously (Park, 2005). Serotomide (trans-*N*-caffeoylserotonin) and safflomide (trans-*N*-caffeoyltryptamine) were prepared with more than 96% purity by HPLC (Waters, Milford, MA), for the experiments.

Cell culture conditions

Renal epithelial (OK) cells were cultured in minimum essential medium (Eagle) with 10% fetal bovine serum. Cell viability was determined microscopically by trypan blue exclusion, and the number of cells was counted by a hematocytometer.

cAMP measurement

Forskolin (100 μM) was added to cultures containing renal epithelial (OK) cells (1×10^6) for 15 min, to induce cAMP production. Serotonin agonists or antagonists were added just prior to adding forskolin (Pauwels and Palmier, 1994). cAMP production was determined in renal epithelial (OK) cells using Correlate-EIA^{TM} Direct cyclic AMP kit (Assay Designs Inc., Ann Arbor, MI). All measurements were performed according to the kit's protocol. Briefly, the cells were treated with 0.1 M HCl. The treated cell samples and cAMP covalently attached to an alkaline phosphatase molecule were simultaneously incubated in the well coated with the antibody, the excess reagents are washed away, and *p*-nitrophenyl phosphate was added. After 5 min incubation, the reaction was stopped and the yellow color generated read on a microplate reader at 405 nm. The measured optical density was used to calculate the concentrations of cAMP.

Statistical analysis

Treatments effects on the parameters measured were compared by analyzing the means for differences using either ANOVA or ANOVA by ranks as appropriate. Differences were considered to be significant when $p < 0.05$. Data points represent the mean ± SD of three or more samples.

Results

The effects of serotomide on forskolin-stimulated cAMP formation in the OK cells

Forskolin is a cell-permeable diterpenoid possessing adenylyl cyclase activating properties (Pauwels and Palmier, 1994; Zgombick and Branchek, 1998). The activation

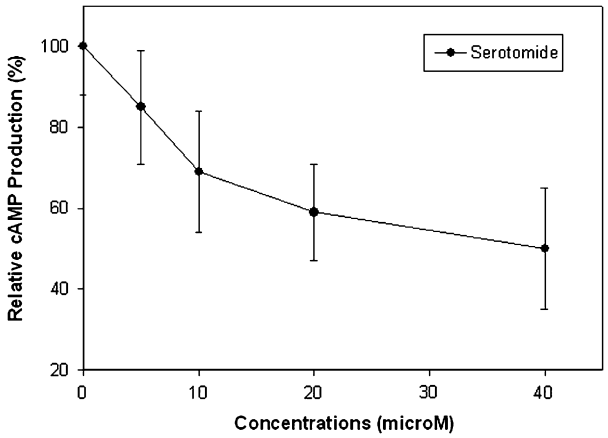


Fig. 2. The effects of serotomide on forskolin-stimulated cAMP production in the OK cells. Renal epithelial (OK) cells (1×10^6) were treated with serotomide for 10 min, prior to adding forskolin (100 μM) for 15 min. cAMP production was measured as described in "Materials and methods". Data points in all figures represent the mean ± SD of more than five samples.

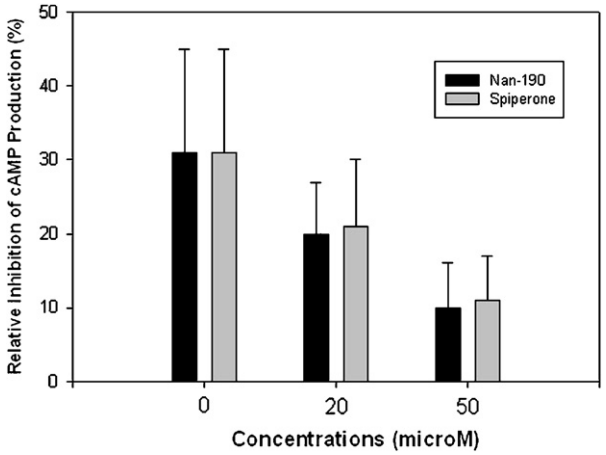


Fig. 3. The effects of 5-HT receptor antagonists on serotomide-suppressed cAMP production in the OK cells. Renal epithelial (OK) cells (1×10^6) were treated with 5-HT antagonists, prior to the treatment of serotomide. cAMP production was induced by forskolin and measured as described in "Materials and methods". Data points in all figures represent the mean ± SD of four samples.

of adenylyl cyclase by forskolin results in the increase of intracellular cAMP concentration (Zgombick and Branchek, 1998; Majumdar et al., 2006). In renal epithelial (OK) cells, 5-HT1 receptors are negatively coupled to adenylate cyclase, thus 5-HT1 receptor agonists decrease cAMP formation stimulated by forskolin via inhibiting adenylate cyclase (Majumdar et al., 2006). At the concentration of 10 μM, serotomide was able to inhibit forskolin-stimulated cAMP formation in the OK cells by 31% ($p < 0.019$). As shown in Fig. 2, the inhibition was positively correlated to the concentrations of serotomide. The data suggest that serotomide can inhibit forskolin-stimulated cAMP formation in renal epithelial (OK) cells expressing 5-HT1 receptors.

The effects of serotonin receptor antagonists on serotomide-suppressed cAMP formation in the OK cells

As demonstrated above, serotomide was able to suppress forskolin-stimulated cAMP formation. However, it is still uncertain that the inhibition of cAMP production ensues via 5-HT1 receptors, negatively coupled to adenylate cyclase in OK cells (Albert and Tiberi, 2001; Odagaki and Toyoshima, 2005; Della Rocca et al., 1999). Previously, it is demonstrated that 5-HT1 receptor antagonists are capable of repressing the inhibition of adenylate cyclase by 5-HT1 receptor agonists (Della Rocca et al., 1999). Therefore, if the inhibition of forskolin-stimulated cAMP production by serotomide occurs via binding to 5-HT1 receptors in the OK cells, serotonin receptor antagonists are able to repress the inhibition. In this experiment, two 5-HT1 receptor antagonists (Nan-190 and spiperone) were used to confirm that serotomide is able to suppress forskolin-stimulated cAMP formation via 5-HT1 receptors in OK cells. As shown in Fig. 3, at the concentrations of 20 μM, both Nan-190 and spiperone repressed the inhibition of forskolin-stimulated cAMP production by serotomide in the OK cells by 33% ($p < 0.013$) and 30% ($p < 0.015$), respectively. The inhibition was further repressed when treated with the high concentration (50 μM) of the two serotonin antagonists (Fig. 3). These data indicate that serotomide may inhibit forskolin-stimulated cAMP production via binding to 5-HT1 receptors on OK cells.

The effects of safflomide on forskolin-stimulated cAMP formation

Because safflomide and serotomide are very similar in their chemical structures, and because serotomide is able to inhibit forskolin-stimulated cAMP formation via activating 5-HT1 serotonin receptors, similar experiments were performed using safflomide to determine whether safflomide can also inhibit forskolin-stimulated cAMP formation. Surprisingly, at the concentration of 10 μM, safflomide could not inhibit forskolin-stimulated cAMP production in OK cells (Fig. 4A), but repress the inhibition of the cAMP by 5-HT receptor agonists (serotonin and 8-OH-DPAT) by 31% ($p < 0.018$) and 36% ($p < 0.013$), respectively (Fig. 4B). This observation suggests that safflomide may play a role as a 5-HT1 receptor antagonist and not as an agonist. The repression was concentration-dependent (data not shown here) and the efficacy of safflomide in repressing the inhibition of forskolin-stimulated cAMP was as effective

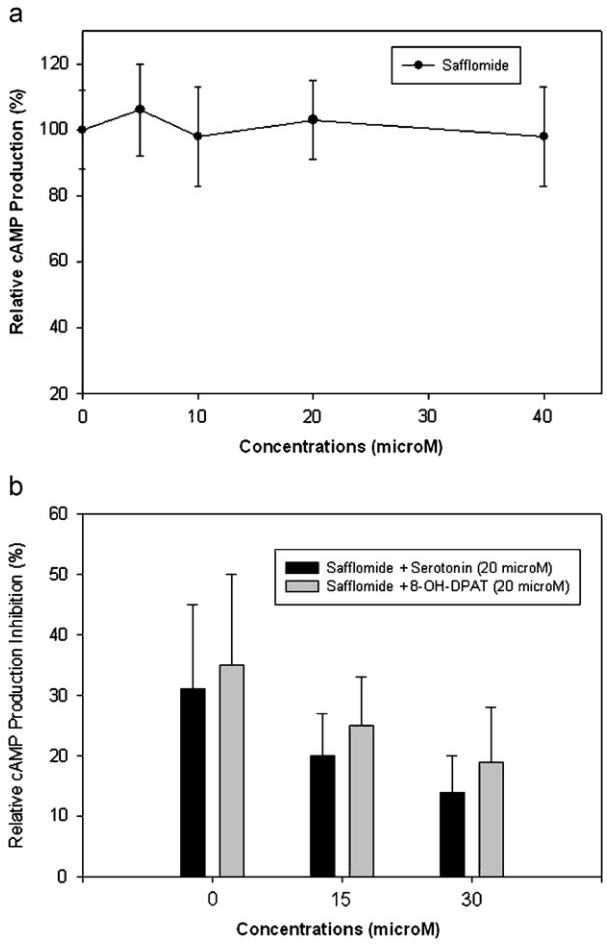


Fig. 4. (A) and (B) the effects of safflomide on forskolin-stimulated cAMP production and 5-HT receptor agonist-suppressed cAMP production. (A) Renal epithelial (OK) cells (1×10^6) were treated with safflomide, prior to the treatment of forskolin. (B) OK cells (1×10^6) were treated with safflomide, prior to the treatment of 5-HT agonists. cAMP production was induced by forskolin and measured as described in "Materials and methods". Data points in all figures represent the mean ± SD of three samples.

as those of Nan-190 and spiperone at the concentration of 20 μM (Fig. 5). These data indicate that 5-hydroxylation in tryptamine moiety play a major role in converting safflomide into the compound able to activate 5-HT1 receptors on OK cells. All together, serotomide and safflomide may act to activate and block 5-HT1 receptors on OK cells, respectively. They may be potent compounds potentially used for treating several human diseases such as anxiety, depression, and cognitive functions treated with 5-HT1 agents.

Discussion

Mental disorders such as depression and anxiety are very common neuropsychiatric conditions that present

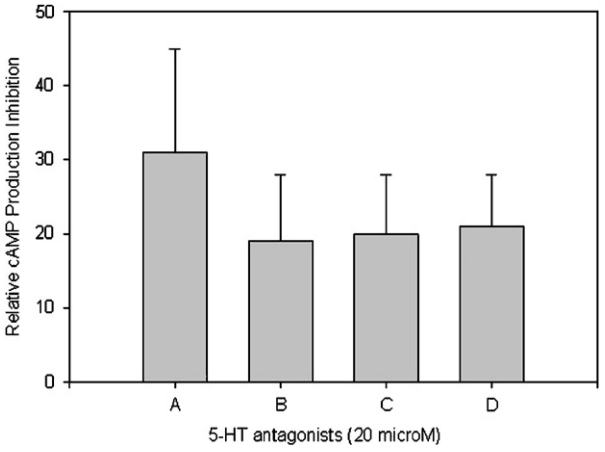


Fig. 5. Comparison of the effects of safflomide, Nan-190 and spiperone on 5-HT receptor agonists-suppressed cAMP production. (A) Control, (B) safflomide, (C) Nan-190, (D) spiperone. Renal epithelial (OK) cells (1×10^6) were treated with 5-HT antagonists (safflomide, Nan-190, spiperone), prior to the treatment of 5-HT agonist serotonin. cAMP production was measured as described in "Materials and methods". Data points in all figures represent the mean ± SD of five samples.

serious health problems in the general population. Since serotonin receptors are believed to be involved in pathophysiologcal conditions of depression and anxiety, these diseases are currently treated with tricyclic antidepressants, selective and non-selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and others. Although therapeutic agents for treating depression and anxiety undoubtedly help alleviate serious conditions derived from the diseases, there are still a great number of studies pointing to reported numerous adverse effects and gradual loss of efficacy of the drugs (Wernicke, 2004; Tomaselli and Modestin, 2004). Therefore, alternative treatments have been sought for years. Traditionally, several plants such as *Hypericum peforatum*, *Passiflora incarnate*, *Schinus molle*, *Rhodiola rosea*, *Crocus sativus*, *Gastrodia elata*, *Piper methysticum*, and *Valeriana officinalis* have been used to treat anxiety and/or depression (Sánchez-Mateo et al., 2007; Miyasaka et al., 2006, 2007; Machado et al., 2007; Akhondzadeh et al., 2007; Perfumi and Mattioli, 2007; Zhou et al., 2006). The efficacy, however, is still uncertain and the mechanisms underlying these herbal remedies are unknown or their purported effects on the diseases poorly defined. In this paper, safflomide-type phenylpropanoid amides (serotomide and safflomide) have been investigated to determine their effects on serotonin receptors on human cells, because of their chemical similarity to serotonin receptor agents used for treating depression, anxiety, and cognitive dysfunction (Lanfumey and Hamon, 2004; Leysen, 2004; Thompson and Lummis, 2006; Hegde and Eglen, 1996; Nelson, 2004). In fact, safflomide-type phenylpropanoid amides can be found in plants such as *Coffea canephora*,

Theobroma cacao, and *Carthamus tinctorius*. Interestingly, these plants-derived products are believed to have beneficial effects on several human diseases such as cardiovascular disease, cognitive dysfunctions, and inflammation. Although, several phytochemicals from plants have been investigated related to human diseases, little is known about the effect of safflomide-type phenylpropanoid amides on serotonin receptors. Therefore, in this study, serotomide and safflomide were investigated and demonstrated to be as potent as pharmacologic compounds used as 5-HT1 receptor ligands. Clinically, 5-HT1 agents have been used for treating several human diseases such as anxiety, depression, and cognitive functions. Therefore, future research should be aimed at determining whether serotomide, safflomide, and their analogues have improved efficacy and fewer adverse effects in treating human diseases associated to 5-HT1 receptors. The preliminary studies indicate that mice fed a standard and defined diet with drinking water containing safflomide (approximately 30 μg/30 g body weight, daily) have not shown any significant change in body weight, food consumption pattern, and daily activities (data not shown here). However, during the course of the studies, behavioral aggressiveness (offensiveness) was observed to be mildly reduced. Several reports indicate that serotonin (5-HT1) receptors are critically involved in modulating aggressive behavior in some animal models (de Boer and Koolhaas, 2005). Therefore, additional studies are currently underway in order to verify the anti-aggressive effects of safflomide using more defined *in vitro* and *in vivo* model systems.

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